http://dx.doi.org/10.35630/2199-885X/2020/10/28

EFFECT OF THE GENETIC VARIABILITY OF METALLOPROTEINASE 9 RS3918242435 GENE POLYMOR-PHISM ON THE COURSE AND OUTCOME OF BRONCHOPULMONARY DYSPLASIA IN CHILDREN

Patimat Khishtilova[1], *Diana Sergienko*, *Stanislav Krasovsky*[2,3]✉

[1] *Astrakhan State Medical University, Astrakhan*
[2] *Research Institute of Pulmonology, Moscow*
[3] *Medical Genetic Research Center, Moscow, Russia*

✉ *sa_krasovsky@mail.ru*

ABSTRACT — The effect of polymorphism in matrix metalloproteinase 9 (MMP-9) rs3918242435 gene on the course and outcome of bronchopulmonary dysplasia (BPD) in children was evaluated. The study proved that despite of association of genotype CC with a short-term respiratory support, it increases the incidence of bronchial obstructive syndrome on the background of the respiratory infections in children, as well as the risk of infection of the respiratory tract with gram-negative microflora and is associated with a long-term therapy with inhaled glucocorticosteroids (ICS).

KEYWORDS — bronchopulmonary dysplasia, children, polymorphism, MMP-9.

INTRODUCTION

In recent years, many studies on the genetic basis of bronchial diseases in children proved the participation of hereditary factors in the development of the disease [3]. As a result of the search for single-nucleotide polymorphic substitutions with the expected phenotypic effect in the promoter and intron regions, a number of MMP gene polymorphisms, particularly polymorphism in metalloproteinase 9 rs3918242435, were identified using bioinformatics methods [2]. As known, MMP-9 (gelatinase-B) is one of the enzymes engaged in remodeling of extracellular matrix, mobilization of matrix-related growth factors, and cytokine processing [1]. To assess the effect of the genetic variability of MMP-9 rs3918242435 gene polymorphism on the course and outcomes of BPD we have analyzed the associations of the clinical manifestations of the disease with polymorphic variants of this gene, with a study of the frequency of the studied polymorphism in samples of patients with bronchopulmonary dysplasia and healthy donors.

MATERIALS AND METHODS

It has been examined 106 patients suffering from bronchopulmonary dysplasia. Molecular genetic studies of the MMP-9 gene polymorphism were carried out by the method of polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). The following parameters in patients with various alleles and genotypes were analyzed: gender, duration and nature of respiratory support, severity of BPD, outcomes of the disease, frequency and severity of bronchial obstruction syndrome, level of treatment, lung function according to bronchophonogram or spirography, sputum culture results, mass growth index, comorbidities. Statistical analysis of the data was carried out using Data Studio software, STATISTICA 6.0 application software package.

RESULTS AND DISCUSSION

During the study, there were no significant differences in the frequencies of C and T alleles and C\C, C\T and T\T genotypes in MMP-9 rs3918242435 polymorphism in the control group and in patients with BPD ($\chi^2 = 5.257$, $p = 0.073$; $df = 2$). When assessing the distribution of alleles and genotypes in relation to severity, statistical significant differences were also not found ($\chi^2 = 389$, ($p = 0.421$); $df = 4$). At the same time the analysis of statistical data showed gender differences in the distribution of genotypes in MMP-9 rs3918242435 polymorphism: C\C genotype was significantly more common in girls in the main group of patients (Table 1).

It was proved that CC genotype of MMP-9 gene polymorphism is predisposing for short-term mechanical ventilation ($\chi^2 = 7.185$ ($p = 0.008$)), $df = 1$ OR = 5,000 (CI 1.574–15.880), at the same time the genotype is protective in regard to long-term mechanical ventilation (more than 1 month) (OR = 0.510 (CI 0.104–0.768)). The study of the effect of MMP-9 rs3918242435 gene polymorphism on outcomes of bronchopulmonary dysplasia in children, particularly, the frequency of recovery or the formation of a chronic bronchopul-monary dysplasia, did not reveal statistically significant differences between C\C, C\T, T\T genotypes ($\chi^2 = 1.820$, ($p = 0.403$), $df = 2$) and also

Table 1. *Distribution of genotype and allele frequencies of MMP-9 rs3918242435 polymorphism with respect to gender in children with BPD*

gender / genotypes	boys (n=76)	girls (n=30)	χ^2 ; p χ^2C\C-T\C-T\T = 7.439; p <0,05; p=0,025; df=2
C\C	56 (73,7%)	28 (93,3%)	χ^2 = 5,049 (p=0,025), df=1 OR=0,200 (CI 0,044–0,917)
C\T	16 (21%)	0	
T\T	4 (5,3%)	2(6,7%)	χ^2 = 0,079 (p=0,779), df=1
alleles	(n=92)	(n=30)	
C	72 (78,3%)	28 (93,3%)	χ^2 = 0,09811 (p=0,063), df=1

between C and T alleles (χ^2 = 1.35 (p = 0.873), df = 1). According to the statistical data, no associations were found between the polymorphism of MMP9 gene and the frequency of acute respiratory viral diseases (ARVI), as well as the first episodes of ARVI up to a year (χ^2 = 0.092, p = 0.955, df = 2).

In accordance with the data in Table 2, the C\C genotype of MMP-9 polymorphism is a predisposing genotype for the development of bronchial obstruction syndrome in children with respiratory infection (χ^2 = 16.133 (p <0.001), df = 1 OR = 7,200 (CI 2.550–20.333)), while the combined effect of C\T and T\T genotypes has a protective effect on this ground (χ^2 = 6.318, p = 0.012; OR = 0.216 (CI 0.071–0.662); df = 1; χ^2 = 7.038, p = 0.008, OR = 0.125 (CI 0.021–0.731).

With the genetic variability of MMP-9 rs3918242435 gene polymorphism, there were statistically significant differences for different genotypes influencing on the nature of the microbiota of the respiratory tract in children with BPD. Gram-negative bacteria (P.aeruginosa, Klebsiella, E. coli) were often found in patients with C\C genotype (χ^2 = 9.708, p = 0.008, df = 2), while the T\T genotype was predisposing to gram-positive bacteria (χ^2 = 9.373, p = 0.010, df = 2). (Table 3)

Analysis of the results showed that C\C genotype is predisposing to prolonged use of ICS in children with BPD, while the representatives of C\T and T\T genotypes have to use ICS in 11.6 and 4.7% of cases (Table 4).

Table 2. *Association of alleles and genotypes of polymorphism of MMP-9 with the development of bronchial obstruction syndrome (BOS)affected by ARVI in children with BPD*

BOS / genotypes	available (n=82)	absent (n=24)	χ^2 ; p χ^2C\C-T\C-T\T = 14,167; (p<0,001); df=2
C\C	72(87,8%)	12 (50%)	χ^2 = 16,133 (p<0,001), df=1 OR=7,200 (CI 2,550– 20,333)
C \T	8 (9,8%)	8 (33,3%)	χ^2 = 6,318 (p=0,012) df=1 OR=0,216 (CI 0,071– 0,662)
T\T	2 (2,4%)	4 (16,7%)	χ^2 = 7,038 (p=0,008), df=1 OR=0,125 (CI 0,021– 0,731)
alleles	n=90	n=32	
C	80(88,9%)	20 (62,5%)	χ^2 = 11,122, (p<0,001), df=1 OR=4,800 (CI 1,816–12,685)

The study of lung function is one of the most important criteria for the diagnosis of respiratory tract diseases when assessing their severity and degree of effectiveness of therapy. According to statistical analysis, no association of the influence of the genotypes and alleles of MMP-9 gene with the level of respiratory tract damage, particularly the upper, middle and lower ones, was detected. (χ^2 = 4.713, p = 0.095; df = 2).

CONCLUSION

The study proved that despite the association of C\C genotype of the matrix metalloproteinase 9 (MMP-9) rs3918242435 gene polymorphism with short-term respiratory support it increases the incidence of bronchial obstructive syndrome affected by respiratory infections in children, the risk of infection of the respiratory tract with gram-negative microflora

Table 3. *Association of genotypes and allele frequencies of MMP-9 rs3918242435 gene polymorphism of microbiota of the bronchial apparatus in children with BPD*

bacteria / genotypes	gram-positive (n=12)	gram-negative (n=50)	No significant microflora (n=44)	χ2 ; p χ2G\G-T\G-T\T= 19,290; p < 0,01; (p<0,001); df=4
C\C	6(50%)	44 (88%)	30(68,2%)	χ2 = 9,708, (p=0,008), df=2
C \T	2 (16,7,6%)	2 (4%)	12 (27,3%)	χ2 = 9,917, (p=0,008), df=2
T\T	4 (33,3%)	4(8%)	2 (4,5%)	χ2 = 9.373 (p=0,010), df=2
alleles	n=14	n=52	n=56	
C	8 (57,1%)	46 (88,5%)	42 (75%)	χ2 = 7,291 (p=0,027), df=2

Table 4.*The effect of MMP-9 rs3918242435 gene polymorphism on the duration of ICS use*

duration / genotypes	constantly (n=86)	episodically (n=20)	χ2 ; p χ2C\C-T\C-T\T = 5,601; p <0,05; (p=0,061); df=2
C\C	72(83,7%)	12 (60%)	χ2 = 5,551; (p=0,019); df=1 OR=3,429 (ДИ 1,185– 9,917)
C \T	10(11,6%)	6 (30%)	χ2 = 4,274 (p=0,039,), df=1
T\T	4 (4,7%)	2 (10%)	χ2 = 0,31609 (p=0,352), df=1
alleles	n=72	n=50	
C	58(80,6%)	42 (84%)	χ2 = 0,81129 (p=0,627), df=1
T	14(19,4%)	8 (16%)	

and is associated with the need for long-term use of ICS. This genotype leads to an increase in the level of MMP which affects the activity of inflammation, the degradation of collagen with the involvement of the neutrophils of inflammation. [1, 2].

Based on the above, the analysis of polymorphism of the metalloproteinase 9 rs3918242435 gene is useful for predicting the course of bronchopulmonary dysplasia in premature infants and the formation of a personalized approach to their treatment.

REFERENCES

1. **Yarmolinskaya M.I., Molotkov A.S., Denisova V.M.** Matrix metalloproteinases and inhibitors: classification, mechanism of action. Journal of Obstetrics and Female Diseases 2012; 61 (1): 113–125.
2. **Pozharischenskaya V.K., Davydova I.V., Savostyanov K.V., Namazova-Baranova L.S., Pavlinova E.B., Pushkov A.V.** Genetic determination of the formation of bronchopulmonary dysplasia: the pros and cons. Pediatric Pharmacology 2017; 14 (1): 24–32.
3. **Ovsyannikov D.Yu.** Bronchopulmonary dysplasia: natural development, outcomes and control / D.Yu. Ovsyannikov // Pediatrics. 2011; 90 (1): 141–150